STIC-ILL

From: Canella, Karen
Sent: Tuesday, May 13, 2003 9:57 PM
To: STIC-ILL
Subject: ill order 09/520,489

Art Unit 1642 Location 8E12(mail)

Telephone Number 308-8362

Application Number 09/520,489

1. Biochemical and Biophysical Research Commun:
   1989, 158(1):155-162
   1991 Jan 31, 174(2):758-766

2. Lymphokine Research, 1989, 8(2):99-106

3. Immunology, 1996 Jan, 87(1):127-133

4. Cancer Immunol Immunother, 1986, 23(1):60-66

5. FEBS Letters, 1995, 372(1):44-48

L2 ANSWER 1 OF 1 MEDLINE
ACCESSION NUMBER: 94259184 MEDLINE
DOCUMENT NUMBER: 94259184 PubMed ID: 7911089
TITLE: pp60v-src kinase overexpression leads to cellular resistance to the antiproliferative effects of tumor necrosis factor.
AUTHOR: Aggarwal B B; Totpal K; Ali-Osman F; Budde R J; Pocsik E
CORPORATE SOURCE: Department of Clinical Immunology and Biological Therapy, University of Texas, M.D. Anderson Cancer Center, Houston 77030.
SOURCE: FEBS LETTERS, **(1994 May 30) 345** (2-3) **219-24.**
Journal code: 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199407
ENTRY DATE: Entered STN: 19940714
Last Updated on STN: 20000303
Entered Medline: 19940706

AB While some tumor cells are sensitive to the antiproliferative effects of tumor necrosis factor (TNF), others are resistant. The molecular basis for cellular resistance to TNF is not completely understood. Previously we have shown that transfection of cells with an oncogene HER2/neu/erb B2, a receptor tyrosine kinase, leads to resistance to the anticellular effects of TNF [(1988) Proc. Natl. Acad. Sci. USA 85, 5102-5106]. In the present study, we demonstrate that the overexpression of another oncogenic tyrosine kinase, pp60v-src also induces resistance to TNF. In contrast to HER2, however, pp60v-src transfection of cells did not lead to down-modulation of TNF receptors but rather to decreased intracellular glutathione levels. The pp60v-src-induced cellular resistance to TNF could be abrogated by interferon-gamma. Thus, these results indicate that the resistance of certain tumors to TNF may also be due in part to the overexpression of pp60v-src oncogene.

L74 ANSWER 13 OF 16 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1991:194298 BIOSIS
DOCUMENT NUMBER: BR40:91578
TITLE: PHASE I-II-TRIAL WITH **TNF-GAMMA** INTERFERON IN **METASTATIC** RENAL CELL CARCINOMA.
AUTHOR(S): SOHN M; LEVENS W; RUEBBEN H; RICHTER R; RAETH U; KEMPENI J;
JAKSE G
CORPORATE SOURCE: DEP. UROLOGY, UNIV. CLINICS RWTH AACHEN, PAUWELSSTRASSE, 5100 AACHEN, W. GER.
SOURCE: 15TH INTERNATIONAL CANCER CONGRESS, HAMBURG, GERMANY, AUGUST 16-22, 1990. J CANCER RES CLIN ONCOL, (1990) 116 (SUPPL PART 2), 1058.
CODEN: JCROD7. ISSN: 0171-5216.
DOCUMENT TYPE: Conference
FILE SEGMENT: BR; OLD
LANGUAGE: English

L46 ANSWER 1 OF 1 PCTFULL COPYRIGHT 2003 Univentio
ACCESSION NUMBER: 1996014328 PCTFULL ED 20020514
TITLE (ENGLISH): **TUMOR NECROSIS FACTOR-GAMMA**
TITLE (FRENCH): POLYPEPTIDE GAMMA APPARTENANT A LA FAMILLE DES FACTEURS DE NECROSE TUMORALE (FNT)
INVENTOR(S): YU, Guo-Liang;
NI, Jian;
ROSEN, Craig, A.
PATENT ASSIGNEE(S): HUMAN GENOME SCIENCES, INC.;
YU, Guo-Liang;
NI, Jian;
ROSEN, Craig, A.
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9614328	A1	19960517

DESIGNATED STATES
W: AU CA CN JP KR NZ US AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
APPLICATION INFO.: **WO 1994-US12880 A 19941107**
TIEN **TUMOR NECROSIS FACTOR-GAMMA**
AI **WO 1994-US12880 A 19941107**
ABEN A human **TNF-gamma** polypeptide and DNA (RNA) encoding such polypeptide and a procedure for producing such polypeptide by recombinant techniques is disclosed.
Also.
. . certain cell types to treat diseases, for example restenosis.
Also
disclosed are diagnostic methods for detecting a mutation in the **TNF-gamma** nucleic acid sequence or an overexpression of the **TNF-gamma** polypeptide. Antagonists against such polypeptides and their use as a therapeutic to treat cachexia, septic shock, cerebral malaria, inflammation, arthritis. . .

DETD The TNF-gamma polypeptide may also be employed to treat autoimmune diseases such as Type I diabetes by enhancing the **T-cell proliferative** response.

L33 ANSWER 3 OF 12 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1997:133820 CAPLUS
DOCUMENT NUMBER: 126:224097
TITLE: Production of prostaglandin E2 and collagenase is inhibited by the recombinant soluble **tumor necrosis factor** receptor p55-human . **gamma**.3 fusion protein at concentrations a hundred-fold lower than those decreasing T cell activation
AUTHOR(S): Nicod, L. P.; Isler, P.; Chicheportiche, R.; Songeon, F.; Dayer, J.-M.
CORPORATE SOURCE: Rekspiratory Div., Univ. Hospital, Geneva, Switz.
SOURCE: European Cytokine Network (**1996**), 7(4), 755-763
CODEN: ECYNEJ; ISSN: 1148-5493
PUBLISHER: Libbey Eurotext
DOCUMENT TYPE: Journal
LANGUAGE: English

AB TNF-.alpha. and lymphotoxin .alpha. (TNF-.beta.) are pleiotropic cytokines with regulatory functions in inflammatory reactions and T cell activation.

Natural TNF inhibitors such as sol. TNF-binding proteins, i.e. TNFsR55 and TNFsR75, are shed from white blood cells and probably other cells. These naturally occurring inhibitors of TNF are shown to be 10 times less effective than the bivalent antagonist of TNF, recombinant sol. TNF receptors p55-human .gamma.3 fusion protein (rsTNFR-p55h.gamma.3), in controlling the release of prostaglandin E2 (PGE2) and collagenase by fibroblasts, as well controlling **T cell proliferation**. To block the action of rhTNF-.alpha. added to fibroblasts, a fivefold excess of rsTNFR-p55h.gamma.3 was sufficient, but concns. of a hundred to a thousand times higher were required to obtain a significant inhibition of T cell activation. This concn. appears to be required to block membrane-bound TNF-.alpha. on peripheral blood mononuclear cells as shown by Scatchard anal. We addnl. show rsTNFR-p55.gamma.3 at high concns. also blocks T cell activation by dendritic cells. In conclusions rsTNFR-p55h.gamma.3 has a much higher anti-inflammatory effect than immunosuppressive effect.

L33 ANSWER 4 OF 12 MEDLINE DUPLICATE 3

L33 ANSWER 2 OF 12 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 97163923 MEDLINE
DOCUMENT NUMBER: 97163923 PubMed ID: 9010678
TITLE: Production of prostaglandin E2 and collagenase is inhibited by the recombinant soluble **tumour necrosis factor** receptor p55-human **gamma** 3 fusion protein at concentrations a hundred-fold lower than those decreasing T cell activation.
AUTHOR: Nicod L P; Isler P; Chicheportiche R; Songeon F; Dayer J M
CORPORATE SOURCE: Respiratory Division, University Hospital, Geneva, Switzerland.
SOURCE: EUROPEAN CYTOKINE NETWORK, **(1996 Dec)** 7 (4) 757-63.
Journal code: 9100879. ISSN: 1148-5493.
PUB. COUNTRY: France
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199703
ENTRY DATE: Entered STN: 19970407
Last Updated on STN: 19970407
Entered Medline: 19970326

AB TNF-alpha and lymphotoxin alpha (TNF-beta) are pleiotropic cytokines with regulatory functions in inflammatory reactions and T cell activation. Natural TNF inhibitors such as soluble TNF-binding proteins, i.e. TNFsR55 and TNFsR75, are shed from white blood cells and probably other cells. These naturally occurring inhibitors of TNF are shown to be 10 times less effective than the bivalent antagonist of TNF, recombinant soluble TNF receptor p55-human gamma 3 fusion protein (rsTNFR-p55h gamma 3), in controlling the release of prostaglandin E2 (PGE2) and collagenase by fibroblasts, as well as in controlling **T cell proliferation**. In order to block the action of rhTNF-alpha added to fibroblasts, a fivefold excess of rsTNFR-p55h gamma 3 was sufficient, but concentrations of a hundred to a thousand times higher were required to obtain a significant inhibition of T cell activation. This concentration appears to be required to block membrane-bound TNF-alpha on peripheral blood mononuclear cells as shown by Scatchard analysis. We additionally show that rsTNFR-p55h gamma 3 at high concentrations also blocks T cell activation by dendritic cells. In conclusion rsTNFR-p55h gamma 3 has a much higher anti-inflammatory effect than immunosuppressive effect.

L38 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:790340 CAPLUS
DOCUMENT NUMBER: 133:355211
TITLE: Death domain-contg. receptor 5 and compns. for treatment of immunity-related diseases, viral diseases, and cancer
INVENTOR(S): Ni, Jian; Gentz, Reiner L.; Yu, Guo-liang; Rosen, Craig A.
PATENT ASSIGNEE(S): Human Genome Sciences, Inc., USA
SOURCE: PCT Int. Appl., 266 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000066156	A1	20001109	WO 2000-US12041	20000504
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1196191	A1	20020417	EP 2000-930329	20000504
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2002543151	T2	20021217	JP 2000-615040	20000504
US 2002072091	A1	20020613	US 2001-874138	20010606

PRIORITY APPLN. INFO.:

US 1999-132498P	P	19990504
US 1999-133238P	P	19990507
US 1999-148939P	P	19990813
US 1997-40846P	P	19970317
US 1997-54021P	P	19970729
US 1998-42583	A1	19980317
US 2000-565009	A1	20000504
WO 2000-US12041	W	20000504

AB The present invention relates to novel Death Domain Contg. Receptor-5 (DR5) proteins which are members of the tumor necrosis factor (TNF) receptor family, and have now been shown to bind TRAIL. In particular, isolated nucleic acid mols. are provided encoding the human DR5 proteins. DR5 polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of DR5 activity, e.g., for treating graft-vs.-host disease, viral infection, cancer, and immune diseases.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L78 ANSWER 17 OF 21 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 97086827 MEDLINE
DOCUMENT NUMBER: 97086827 PubMed ID: 8932856
TITLE: Interferon-gamma plays a key role in the human mixed lymphocyte culture.
AUTHOR: Danzer S G; aCampo C; Rink L
CORPORATE SOURCE: Institute of Immunology and Transfusion Medicine, University of Lubeck School of Medicine, Germany.
SOURCE: BONE MARROW TRANSPLANTATION, (1996 Nov) 18 (5) 991-6. Journal code: 8702459. ISSN: 0268-3369.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199702
ENTRY DATE: Entered STN: 19970306
Last Updated on STN: 19970306
Entered Medline: 19970225

AB Measurement of cytokines in the mixed lymphocyte culture (MLC) is thought to be a new and relevant parameter for bone marrow transplantation (BMT). Our experiments showed that IFN-gamma plays a central role in the cytokine network following alloantigenic recognition. IFN-gamma itself is induced by IL-2 since anti-IL-2 strongly reduced the secretion of IFN-gamma. As anti-IFN-gamma also diminished the response of IL-2 and sIL-2R, a feedback mechanism between these two cytokines is assumed. Addition of rIFN-gamma to the MLC augmented the release of sCD8 molecules, whereas sCD4 molecules were reduced, indicating that IFN-gamma led to T cell differentiation instead of IL-2 dependent proliferation. In the MLC, a feedback mechanism between TNF-alpha and IFN-gamma exists, since **anti-TNF -gamma** reduced the secretion of IFN-gamma and anti-IFN-gamma inhibited the release of TNF-alpha. Therefore, IFN-gamma plays a critical role in monocyte activation, T cell differentiation, and IL-2-induced cell growth. We conclude that measurement of IFN-gamma might be a new and more sensitive parameter for BMT than the established proliferation assay, since IFN-gamma directly quantifies T cell activation.

L78 ANSWER 7 OF 21 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2001-13023 BIOTECHDS
TITLE: Humanization of a mouse monoclonal **antibody** neutralizing **TNF-gamma** by guided selection;
plasmid p3MH expression in Escherichia coli, hybridoma cell culture, phage display
AUTHOR: Wang Z; Wang Y; Li Z; Li J; Dong Z
CORPORATE SOURCE: Navy-Gen.Hosp.Beijing; Univ.Beijing-Med.; Acad.Med.Sci.Beijing
LOCATION: Central Laboratory, Navy General Hospital, Haidian District, Beijing 100037, People's Republic of China.
Email: zwang1@partners.org
SOURCE: J.Immunol.Methods; (2001) 241, 1-2, 171-84
CODEN: JIMMBG
ISSN: 0022-1759
DOCUMENT TYPE: Journal
LANGUAGE: English
AN 2001-13023 BIOTECHDS
AB Humanization of a mouse monoclonal **antibody** neutralizing tumor necrosis factor (**TNF**)-**gamma** by guided selection was discussed. Human recombinant and the hybridoma Z8, which produces a mouse monoclonal **anti-TNF-gamma** **antibody** of **immunoglobulin** (Ig)G1 subclass with the k light chain were used. The isolated Fd and human k genes emerged from guided selection were recloned into the expression vector p3MH and the recombinant vectors were transformed to Escherichia coli TG-1. One of the isolated human Fd genes (huFd2), which showed the strongest reactivity, was chosen to pair with 12 of selected human k chains. Two of the resulting human Fabs (huFad2-huk1 and huFad2-huk2), with same Fd and different k chains, bound to TNF-gamma specificity. Their human origin was proved by ELISA and sequencing analysis. The human Fabs competitive ELISA and in vitro TNF-gamma neutralization assay showed that
the human Fabs resembled its parental mouse mAb Z8 in that they both recognized the same epitope and neutralized the cytotoxicity of TNF-gamma. (32 ref)

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     (FILE 'HOME' ENTERED AT 19:32:50 ON 13 MAY 2003)

     FILE 'MEDLINE, BIOSIS, CANCERLIT, LIFESCI, CAPLUS, BIOTECHDS' ENTERED AT
     19:33:07 ON 13 MAY 2003
L1            14 S TNFG OR GTNF OR TNFGAMMA OR GAMMATNF
L2          3256 S TNF(A)(G OR GAMMA)
L3        251510 S (TUMOR OR TUMOUR)(W)NECROSIS(W)(FACTOR)
L4         17384 S L3(3A)(G OR GAMMA OR FIBROBLAST)
L5             0 S TNF(A)FIBROLAST
L6             0 S TNF(3A)FIBROLAST
L7         19840 S L1 OR L2 OR L4
L8          1560 S L7(5A)(NEUTROPHIL? OR MACROPHAGE# OR MONOCYT?)
L9            17 S L8(5A)(PROLIFER?)
L10           10 DUP REM L9 (7 DUPLICATES REMOVED)
L11         1274 S L7(5A)FIBROBLAST#
L12          875 S L11 AND PY<1998
L13           36 S L11(5A)(PROLIF?(A)FIBROBLAST?)
L14           47 S L11(5A)(PROLIF?(3A)FIBROBLAST?)
L15           26 S L14 AND PY<1998
L16           22 DUP REM L15 (4 DUPLICATES REMOVED)

     FILE 'MEDLINE' ENTERED AT 20:05:24 ON 13 MAY 2003
L17            3 S TNFG OR GTNF OR TNFGAMMA OR GAMMATNF
L18          671 S TNF(A)(G OR GAMMA)
L19        54995 S (TUMOR OR TUMOUR)(W)NECROSIS(W)(FACTOR)
L20         3502 S L19(3A)(G OR GAMMA OR FIBROBLAST)
L21         4023 S L17 OR L18 OR L20
L22            7 S L21(S)(PROLIF?(3A)FIBROBLAST#)
L23          650 S L21(S)(FIBROBLAST# OR (T(W)(CELL# OR LYMPHOCYTE#)))

     FILE 'MEDLINE, BIOSIS, CANCERLIT, LIFESCI, BIOTECHDS, CAPLUS' ENTERED AT
     20:11:19 ON 13 MAY 2003
L24        16242 S L3(3A)(G OR GAMMA)
L25        18700 S L1 OR L2 OR L24
L26           54 S L25(S)(PROLIF?(3A)(FIBROBLAST# OR NEUTROPHIL# OR MONOCYTE# O
L27           38 S L26 AND PY<1998
L28           19 DUP REM L27 (19 DUPLICATES REMOVED)
L29         3123 S L25/TI
L30        72911 S PROLIFER?(3A)(FIBROBLAST# OR NEUTROPHIL? OR MONOCYTE# OR
(T(W
L31           60 S L29 AND L30
L32           50 S L31 AND PY<1998
L33           12 DUP REM L32 (38 DUPLICATES REMOVED)
L34          238 S TNF(W)(G OR GAMMA)
L35          252 S (TUMOR OR TUMOUR)(W)NECROSIS(W)(FACTOR)(W)(G OR GAMMA)
L36          466 S L1 OR L34 OR L35
L37           19 S L36 AND L30
L38           11 DUP REM L37 (8 DUPLICATES REMOVED)

     FILE 'PCTFULL, USPATFULL, EUROPATFULL' ENTERED AT 20:36:00 ON 13 MAY 2003
L39          705 S TNF(W)(G OR GAMMA)
L40          154 S (TUMOR OR TUMOUR)(W)NECROSIS(W)(FACTOR)(W)(G OR GAMMA)
L41           83 S TNFG OR GTNF OR TNFGAMMA OR GAMMATNF
L42        13681 S PROLIFER?(3A)(FIBROBLAST# OR NEUTROPHIL? OR MONOCYTE# OR
(T(W
L43          879 S L39 OR L40 OR L41
L44           34 S L43/TI,AB
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L45           7 S L44 AND L42
L46           1 S L45 AND AD<19970212
L47          79 S L43(S)L42
L48           5 S L47 AND AD<19970212
L49           2 S L48 AND PD>20001129
L50           3 S L48 NOT L49
L51        3491 S YU/IN
L52         422 S NI/IN
L53          57 S L51 AND L52
L54          14 S L53 AND L43
L55           1 S L54 AND AD<19970212
L56           3 S L53 AND (RECEPTOR(W)5)/TI
L57          97 S L43(5A)PROLIF?
L58           3 S L57 AND AD<19970212
L59       44599 S METASTA?
L60        4491 S L59(S)ENDOTHELI?
L61         529 S L60 AND L43
L62           9 S L44 AND L60
L63           0 S L62 AND AD<19970212
L64          63 S L60(S)L43
L65           0 S L64 AND AD<19970212
L66          12 S L61 AND AD<19970212
L67          95 S L60/TI,AB
L68           0 S L67 AND L43
L69          27 S L67 AND AD<19970212

     FILE 'MEDLINE, BIOSIS, CANCERLIT, LIFESCI, BIOTECHDS, CAPLUS' ENTERED AT
     21:22:55 ON 13 MAY 2003
L70        8853 S METASTA?(S)ENDOTHELI?
L71           0 S L70 AND L36
L72      613410 S METASTA?
L73          20 S L72 AND L36
L74          16 DUP REM L73 (4 DUPLICATES REMOVED)
L75           4 S ANTI(W)L36
L76          20 S L36(5A)(ANTIBOD? OR IMMUNOGLOBULIN#)
L77          23 S L75 OR L76
L78          21 DUP REM L77 (2 DUPLICATES REMOVED)
L79           0 S OSTEOCLAST# AND L36
L80           1 S OSTEOBLAST# AND L36

     FILE 'PCTFULL, USPATFULL, EUROPATFULL' ENTERED AT 21:42:44 ON 13 MAY 2003
L81           0 S L44 AND (OSETO?)
L82          17 S L44 AND OSTEO?
L83           2 S L82 AND AD<19970212
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L69 ANSWER 19 OF 27 USPATFULL
ACCESSION NUMBER: 1998:72589 USPATFULL
TITLE: Heparin- and sulfatide binding peptides from the type I repeats of human thrombospondin and conjugates thereof
INVENTOR(S): Roberts, David D., Bethesda, MD, United States
Browning, Philip J., Brentwood, TN, United States
Bryant, Joseph L., Bethesda, MD, United States
Inman, John K., Bethesda, MD, United States
Krutzsch, Henry C., Bethesda, MD, United States
Guo, Nenghua, Gaithersburg, MD, United States
PATENT ASSIGNEE(S): The United States of America as represented by the Department of Health and Human Services, Washington, DC, United States (U.S. government)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5770563		19980623	
APPLICATION INFO.:	US 1995-487568		19950607 (8)	<--

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1994-215085, filed on 21 Mar 1994, now abandoned which is a continuation-in-part of Ser. No. US 1991-801812, filed on 6 Dec 1991, now patented, Pat. No. US 5357041
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Tsang, Cecilia J.
ASSISTANT EXAMINER: Harle, Jennifer
LEGAL REPRESENTATIVE: Townsend and Townsend and Crew, LLP
NUMBER OF CLAIMS: 41
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 64 Drawing Figure(s); 63 Drawing Page(s)
LINE COUNT: 3518
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AI US 1995-487568 19950607 (8) <--
AB . . . motility, extravasation and neovascularization), sulfatides, related sulfated glycoconjugates, fibronectin, and basic fibroblast growth factor, involving malignant cell lines and normal **endothelial** cells. Use of the defined peptides, analogs or peptidomimetics and their conjugates for treatment of **metastatic** tumors, breast carcinomas, melanomas, Kaposi's sarcomas, hemangiomas, diabetic retinopathies, and various pathological conditions dependent upon neovascularization is also disclosed.

L69 ANSWER 20 OF 27 USPATFULL

L69 ANSWER 25 OF 27 USPATFULL
ACCESSION NUMBER: 92:3571 USPATFULL
TITLE: Cloned genes which encode ELAM-1
INVENTOR(S): Bevilacqua, Michael P., Holbrook, MA, United States
Gimbrone, Michael A., Jamaica Plain, MA, United States
Seed, Brian, Boston, MA, United States
Stengelin, Siegfried, Hofheim, Germany, Federal Republic of
PATENT ASSIGNEE(S): Brigham & Women's Hospital, Boston, MA, United States (U.S. corporation)

	NUMBER	KIND	DATE		
PATENT INFORMATION:	US 5081034		19920114		
APPLICATION INFO.:	US 1988-270873		19881114	(7)	<--

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Stone, Jacqueline
ASSISTANT EXAMINER: Low, Christopher S. F.
LEGAL REPRESENTATIVE: Sterne, Kessler, Goldstein & Fox
NUMBER OF CLAIMS: 3
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Figure(s); 2 Drawing Page(s)
LINE COUNT: 717
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AI US 1988-270873 19881114 (7) <--
AB The invention relates to cloned genes, or degenerate variants thereof, which encode **endothelial**-leukocyte adhesion molecule-1 (ELAM-1), or fragments thereof. Such fragments may be leukocyte or complement-binding. The invention also relates to cloned genes. . . also relates to methods for the treatment of inflammation, post reperfusion injury, bacterial infections, vasculitis, leukemia, and methods of inhibiting **metastatic** spread of tumor cells by administering the pharmaceutical compositions of the invention.

ACCESSION NUMBER: 749981 EUROPATFULL EW 200303 FS PS

TITLE: MONOCLONAL ANTIBODY SPECIFIC FOR PD-ECGF / THYMIDINE PHOSPHORYLASE.
PD-ECGF / THYMIDIN PHOSPHORYLASE SPEZIFISCHER MONOKLONALER ANTIKOeRPER.
ANTICORPS MONOCLONAL SPECIFIQUE DU PD-ECGF / THYMIDINE PHOSPHORYLASE.

INVENTOR(S): MIYADERA, Kazutaka, 29-8, Uearatacho, Kagoshima-shi, Kagoshima 890, JP;
YAMADA, Yuji, 2-2953-21, Higashisayamagaoka, Tokorozawa-shi, Saitama 359, JP;
TAKEBAYASHI, Yuji, 5959-24, Kamifukumotocho, Kagoshima-shi, Kagoshima 891-01, JP;
AKIYAMA, Shin-ichi, 3-23-12, Koutokujidai, Kagoshima-shi, Kagoshima 891-01, JP

PATENT ASSIGNEE(S): TAIHO PHARMACEUTICAL CO., LTD., 1-27, Kandanishiki-cho, Chiyoda-ku, Tokyo 101-0054, JP

PATENT ASSIGNEE NO: 332997

AGENT: Waechtershaeuser, Guenter, Prof. Dr., Patentanwalt, Tal 29, 80331 Muenchen, DE

AGENT NUMBER: 12711

OTHER SOURCE: MEPB2003003 EP 0749981 B1 0016

SOURCE: Wila-EPS-2003-H03-T1

DOCUMENT TYPE: Patent

LANGUAGE: Anmeldung in Japanisch; Veroeffentlichung in Englisch; Verfahren in Englisch

DESIGNATED STATES: R AT; R BE; R CH; R DE; R DK; R ES; R FR; R GB; R GR; R IE; R IT; R LI; R LU; R MC; R NL; R PT; R SE

PATENT INFO.PUB.TYPE: EPB1 EUROPAEISCHE PATENTSCHRIFT (Internationale Anmeldung)

PATENT INFORMATION:

PATENT NO	KIND	DATE
EP 749981	B1	20030115
'OFFENLEGUNGS' DATE:		19961227
APPLICATION INFO.:	**EP 1995-941870**	**19951225**
PRIORITY APPLN. INFO.:	JP 1994-327328	19941228
RELATED DOC. INFO.:	WO 95-JP2661	951225 INTAKZ
	WO 96020217	960704 INTPNR
REFERENCE PAT. INFO.:	EP 377855 A	JP 2288897 A

REF. NON-PATENT-LIT.: ASAI K ET AL: "Neurotrophic action of gliostatin on cortical neurons. Identity of gliostatin and platelet - derived endothelial cell growth factor." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 267, no. 28, 5 October 1992 (1992-10-05), pages 20311-6, XP002112454 TOI M ET AL: "Expression of platelet - derived endothelial cell growth factor / thymidine phosphorylase in human breast cancer." INTERNATIONAL JOURNAL OF CANCER, vol. 64, no. 2, 21 April 1995 (1995-04-21), pages 79-82, XP002112676 MOGHADDAM A. ET AL: "Thymidine phosphorylase is angiogenic and promotes tumor growth." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES

OF

AMERICA, vol. 92, no. 4, February 1995 (1995-02), pages 998-1002, XP002112455 TAKEBAYASHI Y ET AL: "Clinicopathological significance of an angiogenic enzyme, thymidine phosphorylase (dThdPase), in

colorectal carcinoma (Meeting abstract)." PROC ANNU MEET AM ASSOC CANCER RES, vol. 36, March 1995 (1995-03), page A535 XP002112456 OGAWA Y ET AL: "An evaluation of thymidine phosphorylase (Dthd Pase) in breast cancer with immunohistochemical staining (Meeting abstract)." PROC ANNU MEET AM ASSOC CANCER RES, vol. 36, March 1995 (1995-03), page A544 XP002112457 J. BIOCHEM., (1993), Vol. 114, No. 1, SUMIZAWA, T. et al., "Thymidine Phosphorylase Activity Associated with Platelet-Derived Endothelial Cell Growth Factor", p. 9-14. CANCER RESEARCH, (1993), Vol. 53, No. 23, HARAGUCHI, M. et al., "Sensitivity of Human KB Cells Expressing Platelet-Derived Endothelial Cell Growth Factor to Pyrimidine Antimetabolites", p. 5680-5682. OSAMU KANEMITSU, "Introduction of Antibody", January 25, 1994, CHIJINSHOKAN, p. 75-144. NATURE, (1992), Vol. 356, FURUKAWA, T. et al., "Angiogenic Factor", p. 668. NATURE, (1989), Vol. 338, ISHIKAWA, F. et al., "Identification of Angiogenic Activity and the Cloning and Expression of Platelet-Derived Endothelial Cell Growth Factor", p. 557-562

AI **EP 1995-941870 19951225**

AI **EP 1995-941870 19951225**

ABEN A monoclonal antibody against a peptide which occurs in human thymidine phosphorylase and vascular **endothelial** cell growth factor originating in human platelets and contains amino acid sequences of SEQ ID NOS: 1 and 2; and a method of immunoassay of human thymidine phosphorylase and/or vascular **endothelial** cell growth factor originating in human platelet by using this monoclonal antibody. The monoclonal antibody recognizes human thymidine phosphorylase and vascular **endothelial** cell growth factor originating in human platelet and is useful in the diagnosis and treatment of various tumors, **metastasis** thereof, diseases accompanied by abnormal vascularization, etc.

L16 ANSWER 19 OF 22 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1989:93274 CAPLUS
DOCUMENT NUMBER: 110:93274
TITLE: Suppression of TNF-stimulated proliferation of diploid fibroblasts and TNF-induced cytotoxicity against transformed fibroblasts by TGF-.beta.
AUTHOR(S): Kamijo, Ryutaro; Takeda, Ken; Nagumo, Masao; Konno, Kunio
CORPORATE SOURCE: Sch. Med., Showa Univ., Tokyo, 142, Japan
SOURCE: Biochemical and Biophysical Research Communications (**1989**), 158(1), 155-62
CODEN: BBRCA9; ISSN: 0006-291X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Human transforming growth factor-.beta. (TGF-.beta.) concn.-dependently inhibited proliferation of WI-38 cells, normal human diploid fibroblasts, stimulated by tumor necrosis factor (TNF). Inhibition occurred at 1 ng/mL concn. of TGF-.beta.. Also, TGF-.beta. dose-dependently suppressed cytotoxicity of TNF against L-929 cells, murine transformed fibroblasts. The concn. of TNF required for 50% cytolysis of L-929 cells was changed from 30 ng/mL to 350 ng/mL by 10 ng/mL TGF-.beta.. This suppression was abolished when L-929 cells were treated with actinomycin D or cycloheximide, suggesting that TGF-.beta. might inhibit the action of TNF via de novo protein synthesis. This response was not due to down regulation of TNF receptor nor to alteration of the affinity of TNF for its receptor.

L16 ANSWER 20 OF 22 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1989:405618 CAPLUS
DOCUMENT NUMBER: 111:5618
TITLE: Modulation of mitogenic activity of tumor necrosis factor by interferons and dexamethasone
AUTHOR(S): Tsujimoto, Masafumi; Sugiyama, Masako; Adachi, Hideki
CORPORATE SOURCE: Suntory Inst. Biomed. Res., Mishima, 618, Japan
SOURCE: Lymphokine Research (**1989**), 8(2), 99-106
CODEN: LYREDH; ISSN: 0277-6766
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Effect of interferon (IFN) on fibroblast growth enhancing activity of tumor necrosis factor (TNF) was examd. IFN-.gamma. and -.beta. inhibited TNF-mediated growth stimulation of FS-4 cells. IFNs also inhibited dexamethasone (DEX)-mediated amplification of mitogenic activity of TNF. Significant inhibition was still demonstrable when IFN-.gamma. was added 2 days after treatment with TNF. On the other hand, no mitogenic activity of TNF was obsd. when cells were pretreated with IFN-.gamma. for 6 h. These results suggested that interaction between TNF and IFN-.gamma. might play a role in modulation of some inflammatory processes in vivo.

L38 ANSWER 9 OF 11 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 96264103 MEDLINE
DOCUMENT NUMBER: 96264103 PubMed ID: 8666424
TITLE: Effect of human cytokines (IFN-gamma, TNF-alpha, IL-1 beta,
IL-4) on porcine endothelial cells: induction of MHC and adhesion molecules and functional significance of these changes.
AUTHOR: Batten P; Yacoub M H; Rose M L
CORPORATE SOURCE: Heart Science Centre, National Heart and Lung Institute at Harefield Hospital, Middlesex, UK.
SOURCE: IMMUNOLOGY, (1996 Jan) 87 (1) 127-33.
Journal code: 0374672. ISSN: 0019-2805.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199608
ENTRY DATE: Entered STN: 19960819
Last Updated on STN: 19970203
Entered Medline: 19960807

AB Previous studies using cultured human endothelial cells have demonstrated the role of inflammatory cytokines [interferon-gamma (IFN-gamma), **tumour necrosis factor-gamma** (TNF-alpha), interleukin-1 beta (IL-1 beta) and IL-4] in the regulation of major histocompatibility complex (MHC) and adhesion molecule expression. These cytokines are therefore implicated in the amplification of allograft, and more recently xenograft, rejection. In view of the likely event of grafted porcine tissues being exposed to human cytokines, we have investigated the effect of IFN-gamma, TNF-alpha, IL-1 beta, IL-4 and recombinant porcine IFN-gamma (rpoIFN-gamma) on cultured porcine aortic endothelial cells (PAEC) with respect to induction/up-regulation of porcine MHC and adhesion molecules and B7 receptors. Expression was detected using monoclonal antibodies (mAb) against porcine ligands and human CTLA-4-immunoglobulin; binding was analysed by flow microfluorimetry. TNF-alpha but not the other human cytokines unregulated swine leucocyte antigens (SLA) class I, class II and B7 receptor expression and induced vascular cell adhesion molecule (VCAM) and E-selectin expression. Porcine IFN-gamma also up-regulated SLA class I and class II, the ligand for CTLA-4-immunoglobulin and VCAM expression; the magnitude and kinetics of this response differed to that produced by recombinant human TNF-alpha (rhTNF-alpha). The ability of untreated, rpoIFN-gamma- and rhTNF-alpha-treated PAEC to stimulate CD4+ T cell was compared. CD4+ **T-cell proliferation** and IL-2 production were significantly enhanced by rhTNF-alpha and rpoIFN-gamma, rpoIFN-gamma being more effective than rhTNF-alpha. Use of blocking antibodies and CTLA-4-immunoglobulin demonstrated that the enhanced proliferative response, but not apparently IL-2 production, was dependent on cytokine-mediated up-regulation of SLA class II and B7 receptors. In conclusion, human TNF-alpha acts as a proinflammatory cytokine on PAEC and is likely to enhance the cellular response to xenogeneic organs in vivo.

L16 ANSWER 22 OF 22 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1987:67178 BIOSIS
DOCUMENT NUMBER: BA83:35504
TITLE: SUPPRESSION OF BOTH MACROPHAGE-MEDIATED TUMOR CELL LYSIS AND CYTOLYTIC FACTOR PRODUCTION BY A FACTOR CYTOLYTIC INHIBITORY FACTOR DERIVED FROM NORMAL EMBRYONIC FIBROBLASTS.
AUTHOR(S): GALLILY R; GIFFORD G E; LOEWENSTEIN J
CORPORATE SOURCE: LAUTENBERG CENTER GENERAL TUMOR IMMUNOLOGY, HEBREW-UNIV. HADASSAH MED. SCH., JERUSALEM 91010, ISR.
SOURCE: CANCER IMMUNOL IMMUNOTHER, (1986) 23 (1), 60-66.
CODEN: CIIMDN. ISSN: 0340-7004.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB We had previously established a murine bone marrow-derived cell line, designated JBM.PHI.1.1, which displayed properties of normal macrophages, including the ability to perform macrophage-mediated cytolysis. It was also found that these cells could be induced by lipopolysaccharide (LPS) to produce reproducibly high levels of a cytolytic factor (CF) resembling tumor necrosis factor (TNF). This cell line was therefore selected for further studies on macrophage-mediated tumor cell lysis and CF production.

Moreover, the CF production during incubation with LPS was higher in the absence of serum than in its presence, with a maximum at days 2-3 following the addition of LPS. A factor inhibitory to CF production (CIF) was detected in our laboratory in the supernatant of embryonic fibroblast cultures. We established the experimental conditions required for the optimal production and supressive effect of CIF. High levels of CIF activity were obtained under conditions that promote fibroblast proliferation. Addition of embryonic fibroblast culture supernatant to the

macrophages shortly before LPS suppressed both LPS-induced CF production and tumoricidal activity. CIF did not affect macrophage protein synthesis in the presence or absence of LPS. However, LPS-induced interleukin 1 release was partially (55%) suppressed by embryonic fibroblast culture supernatant. Our result show that CIF does not exert a general inactivating effect on the macrophages, although it may possibly affect other functions in addition to CF production and tumor cell lysis. The strong inhibition of both the latter properties further indicates that TNF-like CF is an important mediator in macrophage-mediated tumor cell lysis.

L16 ANSWER 11 OF 22 MEDLINE DUPLICATE 1

ACCESSION NUMBER: 91128413 MEDLINE
DOCUMENT NUMBER: 91128413 PubMed ID: 1993070
TITLE: Prostaglandins antagonize **fibroblast proliferation** stimulated by **tumor necrosis factor.**
AUTHOR: Hori T; Yamanaka Y; Hayakawa M; Shibamoto S; Tsujimoto M; Oku N; Ito F
CORPORATE SOURCE: Department of Biochemistry, Faculty of Pharmaceutical Sciences, Setsunan University, Osaka, Japan.
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, **(1991 Jan 31)** 174 (2) 758-66.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199103
ENTRY DATE: Entered STN: 19910405
Last Updated on STN: 19970203
Entered Medline: 19910308

AB Tumor necrosis factor (TNF) is known to be a mitogen for human diploid FS-4 fibroblasts. We have shown in an earlier study (Hori et al. (1989) J. Cell. Physiol. 141, 275-280) that indomethacin further enhances the cell proliferation stimulated by TNF. Since indomethacin inhibits the activity of cyclooxygenase, the role of prostaglandins in TNF-stimulated cell growth was examined. Cell growth stimulated by TNF and indomethacin was inhibited by exogenously added prostaglandins (PGE2, PGF2 alpha, and PGD2), among which PGE2 caused the greatest inhibition of cell growth. Treatment of FS-4 cells with 10 ng/ml TNF resulted in the release of prostaglandins (PGE2, 6-keto-PGF1 alpha, PGA2, PGD2, and PGF2 alpha) 2 to 4 fold over that of untreated cells. The amount of all these prostaglandins increased in a time-dependent manner over 6 h after treatment. In both TNF-treated and control cells, PGE2 was released as the predominant prostaglandin. Furthermore, when PGE2 production and DNA synthesis were determined in FS-4 cells treated with increasing doses of indomethacin, these two cellular responses were inversely affected by indomethacin. These data show that prostaglandins induced by TNF antagonize growth stimulatory action of TNF.

L16 ANSWER 5 OF 22 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1995:847142 CAPLUS
DOCUMENT NUMBER: 124:199946
TITLE: Transfection of cells with basic fibroblast growth factor and Kaposi fibroblast growth factor genes induce resistance to and receptor modulation of tumor necrosis factor
AUTHOR(S): Aggarwal, Bharat B.; Pocsik, Eva; Totpal, Klara
CORPORATE SOURCE: Cytokine Research Laboratory, Department of Molecular Oncology, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX, 77030, USA
SOURCE: FEBS Letters (**1995**), 372(1), 44-8
CODEN: FEBLAL; ISSN: 0014-5793
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Tumor necrosis factor (TNF) has been shown to inhibit the growth of some cell types and stimulate the proliferation of others by a mechanism that is not understood. In the present study, the authors investigated the effect of transfection of NIH-3T3 cells with either the basic fibroblast growth factor gene (bFGF) or the Kaposi FGF gene (K-fgf) on the growth-modulatory effects of TNF. The results show that transformation of cells with either gene leads to resistance to the growth-inhibitory effects of TNF. The K-fgf gene was a more potent inducer of cellular resistance than the bFGF gene. The cellular resistance correlated with the inhibition of TNF-induced activation of phospholipase A2 and down-modulation of TNF receptors. Overall, the results indicate that both K-fgf and bFGF play an important role in suppression of antiproliferative effects of TNF.

L10 ANSWER 10 OF 10 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1990:75020 CAPLUS
DOCUMENT NUMBER: 112:75020
TITLE: Stimulation of polymorphonuclear neutrophils by TNF (tumor necrosis factor) muteins
AUTHOR(S): Kamijo, Ryutaro; Takeda, Ken; Konno, Kunio; Nagumo, Masao; Hasegawa, Akira; Inaka, Koji; Ikehara, Morio
CORPORATE SOURCE: Sch. Med., Showa Univ., Japan
SOURCE: Ensho (1989), 9(1), 25-8
CODEN: ENSHEE; ISSN: 0389-4290
DOCUMENT TYPE: Journal
LANGUAGE: Japanese

AB Tumor necrosis factor (TNF) plays an important role in inflammatory responses other than inducing hemorrhagic necrosis of animal tumors or exhibiting cytotoxicity to tumor cells. Five recombinant human TNF muteins (amino acid sequence partly changed by protein engineering techniques) were prepd. and their biol. activity in stimulating polymorphonuclear neutrophil functions were compared by measuring iodination activity. TNF (C-Phe) (leucine changed to phenylalanine in C-terminal) was more potent than the parent TNF in activating polymorphonuclear neutrophils, although the binding activity of TNF (C-Phe) to neutrophil membrane receptor was less than that of the parent TNF. Other TNF muteins also showed this activity in parallel with their receptor binding activity to neutrophils. The stimulating activity of TNF muteins on polymorphonuclear function was proportional to the proliferation-enhancing activity on fibroblasts.

ACCESSION NUMBER: 522606 EUROPATFULL EW 199614 FS PS

TITLE: Pyridine derivatives, their production and use.
Pyridinderivate, deren Herstellung und Anwendung.
Derives de pyridine, leur preparation et utilisation.

INVENTOR(S): Takatani, Muneo, 6-1 Taniguchisono-machi, Ukyo-ku, Kyoto 616, JP;
Saijo, Taketoshi, 5-9 Fushiodai 2-chome, Ikeda, Osaka 563, JP;
Tomimatsu, Kiminori, E-104, 7 Nyoidani 3-chome, Minoo, Osaka 562, JP

PATENT ASSIGNEE(S): TAKEDA CHEMICAL INDUSTRIES, LTD., 1-1, Doshomachi 4-chome, Chuo-ku, Osaka 541, JP

PATENT ASSIGNEE NO: 204706

AGENT: von Kreisler, Alek, Dipl.-Chem. et al, Patentanwaelte von Kreisler-Selting-Werner Postfach 10 22 41, D-50462 Koeln, DE

AGENT NUMBER: 12434

OTHER SOURCE: EPB1996023 EP 0522606 B1 960403

SOURCE: Wila-EPS-1996-H14-T1

DOCUMENT TYPE: Patent

LANGUAGE: Anmeldung in Englisch; Veroeffentlichung in Englisch

DESIGNATED STATES: R AT; R BE; R CH; R DE; R DK; R ES; R FR; R GB; R GR; R IT; R LI; R LU; R NL; R PT; R SE

PATENT INFO.PUB.TYPE: EPB1 EUROPAEISCHE PATENTSCHRIFT

PATENT INFORMATION:

PATENT NO	KIND	DATE
EP 522606	B1	19960403
'OFFENLEGUNGS' DATE:		19930113
APPLICATION INFO.: EP 1992-201288		19920507
PRIORITY APPLN. INFO.: JP 1991-105691		19910510
REFERENCE PAT. INFO.: EP 103503	A	EP 177907 A
US 3687959	A	US 4786644 A

REF. NON-PATENT-LIT.: CHEMICAL ABSTRACTS, vol. 85, no. 14, 4 October 1976, Columbus, Ohio, US; abstract no. 99178d, G. KALOPISSIS ET AL. 'Cysteamine derivatives for oral treatment of seborrhea.' page 325 CHEMICAL ABSTRACTS, vol. 61, no. 1, 6 July 1964, Columbus, Ohio, US; abstract no. 638g, I.KH. FEL DMAN ET AL. 'Synthesis in the pyridine series. III. Synthesis of quaternary derivatives of beta-aminoethyl pyridyl sulfides'

L66 ANSWER 2 OF 12 PCTFULL COPYRIGHT 2003 Univentio
ACCESSION NUMBER: 1996001653 PCTFULL ED 20020514
TITLE (ENGLISH): METHODS AND COMPOSITIONS FOR THE SPECIFIC COAGULATION OF VASCULATURE
TITLE (FRENCH): PROCEDES ET COMPOSITIONS POUR LA COAGULATION SPECIFIQUE DE VAISSEAUX
INVENTOR(S): THORPE, Philip, E.; EDGINGTON, Thomas, S.
PATENT ASSIGNEE(S): BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM; THE SCRIPPS RESEARCH INSTITUTE; THORPE, Philip, E.; EDGINGTON, Thomas, S.
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9601653	A1	19960125

DESIGNATED STATES
W: AM AT AU BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU IS JP KE KG KP KR KZ LK LR LT LU LV MD MG MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TT UA US UZ VN KE MW SD SZ UG AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
APPLICATION INFO.: **WO 1995-US7439 A 19950607**
PRIORITY INFO.: US 1994-8/273,567 19940711
AI **WO 1995-US7439 A 19950607**

DETD Exemplary inducible antigens include those inducible by a cytokine, e.g., IL-1, IL-4, TNF-u, **TNF-g** or IFN-T, as may be released by monocytes, macrophages, mast cells, helper T cells, CD8-positive T-cells, NK cells or even tumor cells. Examples. . .

The induction of tumor infarction by targeting coagulation-inducing proteins to these and other tumor **endothelial** cell markers is proposed as a valuable new approach to the treatment of solid tumors. The coupling of human (or humanized) antibodies. . . wholly human coaguligands is particularly contemplated, thus permitting repeated courses of treatment to be given to combat both the primary tumor and its **metastases**.

L55 ANSWER 1 OF 1 PCTFULL COPYRIGHT 2003 Univentio
ACCESSION NUMBER: 1996014328 PCTFULL ED 20020514
TITLE (ENGLISH): **TUMOR NECROSIS FACTOR-GAMMA**
TITLE (FRENCH): POLYPEPTIDE GAMMA APPARTENANT A LA FAMILLE DES FACTEURS DE NECROSE TUMORALE (FNT)
INVENTOR(S): **YU, Guo-Liang;**
NI, Jian;
ROSEN, Craig, A.
PATENT ASSIGNEE(S): HUMAN GENOME SCIENCES, INC.;
YU, Guo-Liang;
NI, Jian;
ROSEN, Craig, A.
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9614328	A1	19960517

DESIGNATED STATES
W: AU CA CN JP KR NZ US AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
APPLICATION INFO.: **WO 1994-US12880 A 19941107**
TIEN **TUMOR NECROSIS FACTOR-GAMMA**
IN **YU, Guo-Liang;**
NI, Jian;
ROSEN, Craig, A.
AI **WO 1994-US12880 A 19941107**
ABEN A human **TNF-gamma** polypeptide and DNA (RNA) encoding such polypeptide and a procedure for producing such polypeptide by recombinant techniques is disclosed.
Also.
. . . certain cell types to treat diseases, for example restenosis.
Also
disclosed are diagnostic methods for detecting a mutation in the **TNF-gamma** nucleic acid sequence or an overexpression of the **TNF-gamma** polypeptide. Antagonists against such polypeptides and their use as a therapeutic to treat cachexia, septic shock, cerebral malaria, inflammation, arthritis. . .

DETD . . . has been identified as a novel member of the TNF family based on structural, amino acid sequence homology, and functional similarities, for example, **TNF-gamma** is a pro-inflammatory protein.

In accordance with one aspect of the present invention, there is provided a novel mature polypeptide which is **TNF-gamma**, as well as biologically active and diagnostically or therapeutically useful fragments, analogs and derivatives thereof. The polypeptide of the present invention is. . .

in accordance with another aspect of the present invention, there are provided isolated nucleic acid molecules

encoding human **TNF-gamma**, including mRNAs, DNAs, cDNAs,
genomic DNAs as well as analogs and biologically active and diagnostically or therapeutically useful fragments and derivatives thereof.

. . .

there is provided a process for producing such polypeptide by recombinant techniques comprising culturing recombinant prokaryotic and/or eukaryotic host cells, containing a human **TNF-gamma** nucleic acid sequence, under
conditions promoting expression of said protein and subsequent recovery of said protein.

. . .

present
invention, there are also provided nucleic acid probes comprising nucleic acid molecules of suf f icient length to specifically hybridize to human **TNF-gamma** sequences.

In accordance with another aspect of the present invention, there are provided **TNF-gamma** agonists which mimic
TNF-gamma and binds to the **TNF-gamma** receptors to elicit **TNF-gamma** type responses.

. . .

another aspect of the present
invention, there are provided diagnostic assays f or detecting diseases related to the under-expression and over-expression of the **TNF-gamma** polypeptide and nucleic acid sequences
encoding such polypeptide.

Figure 2 illustrates an amino acid sequence alignment between **TNF-gamma** and other members of the TNF family. **TNF-gamma** contains the conserved amino acid residues of the TNF family as shown by the shaded areas.

Figure 3A is an RNA blot analysis showing the human tissues where **TNF-gamma** is expressed. RNA from the tissues
indicated were probed with labeled **TNF-gamma** cDNA. **TNF-gamma**
MRNA exists predominantly in the kidney since Figure 3A shows a distinct band. Other lanes seem to show strong hybridization, however, these are. . .

Figure 3B is an RNA blot analysis showing that **TNF-gamma**
is expressed predominantly in HUVEC cells (human umbilical vein endothelial cells) which is lane 9. Lane 6 and lane 8 are non-specific smears. RNA from the cell lines indicated were probed with labeled **TNF-gamma** cDNA. Lane 1 is CAMAI
(breast cancer) ; lane 2 AN3CA (uterine cancer) ; lane 3, SK.UT.1 (uterine cancer); lane 4, MG63. . .

Figure 4 is a photograph of a gel after electrophoresing **TNF-gamma** was produced by bacterial expression and purification.

Figure 5 is a photograph of a gel after baculovirus expression of **TNF-gamma**.

Figure 6B illustrates the ability of **TNF-gamma** in comparison to TNF-a and TNF-0 to inhibit WEHI 164 cell growth.

Figure 7 illustrates the ability of recombinant **TNF-gamma**, TNF-a and TNF-0 to induce induce WEHI 164 cell death.

Figure 8 illustrates the ability of recombinant TNF-a, TNF-fl, and **TNF-gamma** to induce morphological change in L929 cells. The morphology change is indicated by dark round cells. Cells were treated with E. Coli. . .

Figure 9 is a graphical illustration of the effect of **TNF-gamma**, TNF-a and TNF-0 on venous endothelial cells. Cell proliferation after venous endothelial cells were treated with commercially available TNF-ce and TNF-0 and E. Coli produced **TNF-gamma** was quantified using an MTS assay.

Figure 10 is a photograph of HL60 cells, with control showing the HL60 cells being spread apart; TNF-a and **TNF-gamma** induce cell adhesion and cell-cell contact as illustrated by the cells adhering together in the lower right.

Figure 11 illustrates that **TNF-gamma** does not significantly bind to two known soluble TNF receptors, namely sTNF RI (p55) and sTNF RII (p75).

Sequences conserved throughout the members of the TNF family are also conserved in **TNF-gamma** (see Figure 2). The bolded letters indicate conserved amino acid residues. The **TNF-gamma** mRNA is specifically expressed in human umbilical vein endothelial cells as shown in the RNA blot analysis of Figure 3B.

The present invention further relates to a **TNF-gamma** polypeptide which has the deduced amino acid sequence of Figure 1 or which has the amino acid sequence encoded by the deposited cDNA,. . .

. . .

etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the **TNF-gamma** genes. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression,. . .

The **TNF-gamma** polypeptides can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation. . .

The **TNF-gamma** polypeptide of the present invention may be employed to inhibit tumor cell growth or neoplasia. The **TNF-gamma** polypeptide may be responsible for tumor destruction throuph apoptosis which is characterized by membrane blebbin (zeiosis) . condensation of cytoplasma and the activation of an endogeneous endonuclease (Figure 7). As shown in Table 1. **TNF-gamma** has strong cytotoxic activity for the cell lines tested which have abnormal cellular proliferation and regulation, for example the fibrosarcoma and carcinoma cell line. This is also illustrated in Figure 6A, 6B and 8 where it is shown that **TNF-gamma** has the ability to inhibit L929 and WEHI 164 cell growth through cytotoxic activity. WEHI 164 cells are mouse fibrosarcoma cells. A preferable method of administering the **TNF-gamma** is by injection directly into the tumor.

The cell adhesion activity of **TNF-gamma** may be employed for wound healing. As shown in Table I and Figure 9, **TNF-gamma** has a strong endothelial cell proliferation effect which is an indication that **TNF-gamma** plays a role in wound healing. **TNF-gamma**'s cell adhesive effects may also play a role in wound healing.

TNF-gamma may also be employed to treat diseases which require growth promotion activity, for example, restenosis.

As stated above, **TNF-gamma** is shown to have strong proliferation effects on endothelial cell growth.

Accordingly, **TNF-gamma** may also be employed to regulate hematopoiesis and endothelial cell development.

The **TNF-gamma** polypeptide, through its ability to stimulate the activation of T-cells, is an important mediator of the immune response. Accordingly, this polypeptide may be used to stimulate an immune response against a variety of parasitic, bacterial and viral infections. **TNF-gamma** may lyse virus-infected cells and, therefore, be employed to arrest HIV infected cells.

The **TNF-gamma** polypeptide may also be employed to

treat autoimmune diseases such as Type I diabetes by enhancing the T-cell proliferative response.

Table 1
Summary of **TNF-gamma** activity
Cell lines Source and Activity
Type
Cyto- Prolif- Differ- Ad-
toxicity eration entiation hesion
L929 mouse fibroblast + -
WEHI 164 mouse
fibrosarcoma ... -
NRK-54E rat. . .

This invention provides a method for identification of the receptor for **TNF-gamma**. The gene encoding the receptor
can be identified by numerous methods known to those of skill in the art, for example, ligand. . . Current Protocols in Immun., 1(2), Chapter
St (1991)). Preferably, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to **TNF-gamma**, and a cDNA library created from this RNA is
divided into pools and used to transfect COS cells or other cells that are not responsive to **TNF-gamma**. Transfected
cells which are grown on glass slides are exposed to labeled **TNF-gamma**. **TNF-gamma** can be
labeled by a variety of means
including iodination or inclusion of a recognition site for a site-specific protein kinase. Following. . .

As an alternative approach for receptor identification, labeled **TNF-gamma** can be photoaffinity linked with cell
membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE and exposed to X-ray film. The labeled complex containing the **TNF-gamma**-receptor can be excised, resolved into peptide
fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to. . .

TNF-gamma does not bind significantly to two soluble TNF
receptors, sTNF-RI (p55) and sTNF-RII (p75). Accordingly, **TNF-gamma** may have activities inclusive of and additional to
known TNF proteins (see Figure 11).

This invention is also related to a method of screening compounds to identify those which mimic **TNF-gamma** (agonists)
or prevent the effect of **TNF-gamma**. An example of such a
method takes advantage of the ability of **TNF-gamma**

to
significantly stimulate the proliferation of human endothelial cells in the presence of the comitogen Con A.

Alternatively, the response of a known second messenger system following interaction of **TNF-gamma** and receptor would
be measured and compared in the presence or absence of the compound. Such second messenger systems include but are. . .

To assay for antagonists, the assay described above is performed, however, in this assay **TNF-gamma** is added along
with the compound to be screened and the ability of the compound to inhibit NIthymidine incorporation in the presence of **TNF-gamma**, indicates that the compund is an
antagonist to **TNF-gamma**. Alternatively, **TNF -gamma**
antagonists may be detected by combining **TNF-gamma** and a
potential antagonist with membrane-bound **TNF-gamma** receptors
or recombinant receptors under appropriate conditions for a competitive inhibition assay. **TNF-gamma** can be labeled, such
as by radioactivity, such that the number of **TNF-gamma** molecules bound to the receptor can determine the effectiveness of the potential antagonist.

Alternatively, a mammalian cell or membrane preparation expressing the **TNF-gamma** receptor is incubated with labeled
TNF-ganima in the presence of the compound. The ability of the compound to enhance or block this. . .

Antibodies specific to TNF-ganina may be used as antagonists by binding to **TNF-gamma** and preventing it from
binding to its receptor. Monoclonal antibodies are particularly effective in this regard. Antibodies specific to the **TNF-gamma** receptor, however, may mediate distinct
cellular responses which tend to agonize the effects of **TNF-gamma** upon interaction with its receptor.

Potential **TNF-gamma** antagonists also include **TNF-gamma**
mutants which bind to the **TNF-gamma** receptor and elicit no
second messenger response to effectively block the receptor from its natural ligand. Specifically designed
oligonucleotides and small molecules may also bind to the **TNF-gamma** receptor and block it from **TNF-gamma**. Examples of
small molecules include but are not limited to small peptides or peptide-like molecules.

Another potential **TNF-gamma** antagonist is a soluble form of the **TNF-gamma** receptor which binds to **TNF-gamma** and prevents it from interacting with membrane-bound **TNF-gamma** receptors. In this way, the receptors are not stimulated by **TNF-gamma**.

Another potential **TNF-gamma** antagonist is an antisense construct prepared using antisense technology. Antisense technology can be used to control gene expression through triple-helix formation or antisense. . . al, Science, 241:456 (1988); and Dervan et al. , Science, 251: 1360 (1991)) , thereby preventing transcription and the production of **TNF-gamma**.

The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into the **TNF-gamma** polypeptide (Antisense - Okano,, J. Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). The oligonucleotides. . . can also be delivered to cells such that the antisense RNA or DNA may be expressed in vivo to inhibit production of **TNF-gamma**.

. . . employed to treat cachexia which is a lipid clearing defect resulting from a systemic deficiency of lipoprotein lipase which is suppressed by **TNF-gamma**. The **TNF-gamma** antagonists are also employed to treat cerebral malaria in which **TNF-gamma** appears to play a pathogenic role. The antagonists may also be employed to treat rheumatoid arthritis by inhibiting **TNF-gamma** induced production of inflammatory cytokines such as IL-1 in the synovial cells. When treating arthritis **TNF-gamma** is preferably injected intra-articularly.

The **TNF-gamma** antagonists may also be employed to prevent graft rejection by preventing the stimulation of the immune system in the presence of a graft by **TNF-gamma** .

The **TNF-gamma** antagonists may also be employed to treat osteoporosis since **TNF-gamma** may induce bone resorption. osteo clasts?

Antagonists to **TNF-gamma** may also be employed as anti-inflammation agents since **TNF-gamma** mediates an enhanced inflammatory response.

Fragments of the full length **TNF-gamma** gene may be used as a hybridization probe for a cDNA library to isolate the full length gene and to isolate other. . . be used to identify a cDNA clone corresponding to a full length transcript and a genomic clone or clones that contain the complete **TNF-gamma** gene including regulatory and promotor regions, exons, and introns. As an example of a screen comprises isolating the coding region of the **TNF-gamma** gene by using the known DNA sequence to synthesize an oligonucleotide probe. Labeled oligonucleotides having a sequence complementary to that of the gene. . .

The **TNF-gamma** polypeptides and agonists and antagonists of the present invention may be employed in combination with a suitable pharmaceutical carrier. Such compositions comprise a. . .

The **TNF-gamma** polypeptides and agonists and antagonists which are polypeptides may also be employed in accordance with the present invention by expression of such. . .

This invention is also related to the use of the **TNF-gamma** gene as part of a diagnostic assay for detecting diseases or susceptibility to diseases related to the presence of mutated **TNF-gamma**. Such diseases are related to an under-expression of **TNF-gamma**, for example, abnormal cellular proliferation such as tumors and cancers.

Individuals carrying mutations in the human **TNF-gamma** gene may be detected at the DNA level by a variety of techniques. Nucleic acids for diagnosis may be obtained from a patient's. . .

As an example, PCR primers complementary to the nucleic acid encoding **TNF-gamma** can be used to identify and analyze **TNF-gamma** mutations. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled **TNF-gamma** RNA or alternatively, radiolabeled **TNF-gamma** antisense DNA sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures.

The present invention also relates to a diagnostic assay for detecting altered levels of **TNF-gamma** protein in various tissues since an over-expression of the proteins compared to

normal control tissue samples may detect the presence of a disease or susceptibility to a disease, for example, tumors and cerebral malaria. Assays used to detect levels of **TNF-gamma** protein in a sample derived from a host are well-known to those of skill in the art and include radioimmunoassays, competitive-binding assays,. . . assay. An ELISA assay (Coligan, et al., Current Protocols in Immunology, 1(2), Chapter 6, (1991)) initially comprises preparing an antibody specific to the **TNF-gamma** antigen, preferably a monoclonal antibody. In addition a reporter antibody is prepared against the monoclonal antibody. To the reporter antibody is attached. . .

Next, the monoclonal antibody is incubated in the dish during which time the monoclonal antibodies attach to any **TNF-gamma** proteins attached to the polystyrene dish. Al 1 unbound monoclonal antibody is washed out with buffer. The reporter antibody linked to horseradish perosxidase is now placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to **TNF-gamma**. Unattached reporter antibody is then washed out. Peroxidase substrates are then added to the dish and the amount of color developed in a given time preiod is a measurement of the amount of **TNF-gamma** protein present in a given volume of patient sample when compared against a standard curve.

A competition assay may be employed wherein antibodies specific to **TNF-gamma** are attached to a solid support and labeled TNF-ganana and a sample derived from the host are passed over the solid support and the amount of label detected, for example by liquid scintillation chromotagraphy, can be correlated to a quantity of **TNF-gamma** in the sample.

A sandwich assay is similar to an ELISA assay. In a sandwich assay **TNF-gamma** is passed over a solid support and binds to antibody attached to a solid support. A second antibody is then bound to the **TNF-gamma**. A third antibody which is labeled and specific to the second antibody is then passed over the solid support and binds to. . .

Example 1
Bacterial Expression and Purification of **TNF-Gamma**
The DNA sequence encoding **TNF-gamma**, ATCC # 75927, is initially amplified using PCR oligonucleotide primers corresponding to the 51 sequences of the **TNF-gamma** protein and the vector sequences 30 to the **TNF-gamma** gene.

Additional nucleotides corresponding to **TNF-gamma** were added

to the 5' and 31 sequences respectively. The 5' oligonucleotide primer has the sequence 50 GCGCGGATCCACCATGAGACGL7.riviTTAAGCAAAGTC 31 contains a Bam HI restriction enzyme site followed by the first 24 nucleotides of **TNF-gamma** coding sequence starting from the presumed terminal amino acid of the processed protein codon. The 3' sequence 51' CGCGTCTAGACTATAGTAAGAAGGCTCCAAAGAAGG 31 contains complementary sequences to an XbaI site and is followed by 22 nucleotides of **TNF-gamma** and to a pQE-9 vector sequence located 31 to the **TNF-gamma** DNA insert. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9 (Qiagen). pQE-9 was then digested. . . Cells were then harvested by centrifugation. The cell pellet was solubilized in the chaotropic agent 6 Molar Guanidine HC1. After clarification, solubilized **TNF-gamma** was purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). **TNF-gamma** was further purified by a second run on the Nickel-chelate column. **TNF-gamma** (90% pure) was eluted from the column in molar guanidine HCl pH 5.0 and for the purpose of renaturation was dialyzed in. . . where M is molecular weight markers; lane I is induced cell lysate; lane 2 is uninduced call lysate; lane 3 is the **TNF-gamma** protein after two Nickel-chelate column purifications; lane 4 is the **TNF-gamma** protein after 1 column purification.

Examnle 2

Cloning and expression of TNF-cramma using the baculovirus expression system

The DNA sequence encoding the full length **TNF-gamma** protein, ATCC 75927, was amplified using PCR oligonucleotide primers corresponding to the 51 and 31 sequences of the gene.

. . .

primer has the sequence 50 GCGCGGATCCACCATGAGACGCTTTTTAAGCAAAGTC 31 and contains a Bam HI restriction enzyme site (in bold) followed by 24 nucleotides of the **TNF-gamma** gene (the initiation codon for translation 11ATG11 is underlined).

. . .

50 CGCGTCTAGACTATAGTAAGAAGGCTCCAAAGAAGG 3' and contains the cleavage site for the restriction endonuclease XbaI and 22 nucleotides complementary to the 31 non-translated sequence of the **TNF-gamma** gene. The amplified sequences were isolated

from a 1% agarose gel using a commercially available kit (Geneclean, 11 BIO 101 Inc. ,. . .

The vector pA2 (modification of pVL941 vector, discussed below) is used for the expression of the **TNF-gamma** protein
using the baculovirus expression system (for review see.

5 Ag of the plasmid pBac **TNF-gamma** was cotransfected with 1.0 Mg of a commercially available linearized baculovirus (BaculoGold baculovirus DNA, Pharmingen, San Diego, CA.) using the lipofection method (Felgner. . .

1Ag of BaculoGold' virus DNA and 5 jig of the plasmid pBac **TNF-gamma** were mixed in a sterile well of a microtiter
plate containing 50 Al of serum free Grace's medium (Life Technologies Inc -. . .
. . .
Sf 9 cells were grown in Grace Is medium supplemented with 10% heat-inactivated FBS. The cells were infected with the recombinant baculovirus V-**TNF-gamma** at a multiplicity of
infection (MOI) of 2. Six hours later the medium was removed and replaced with SF900 II medium minus. . . the labelled proteins visualized by SDS-PAGE and autoradiography. Figure 5 illustrates the gel where Lanes 1 and 3 are the medium of the **TNF-gamma** and control; lanes 2 and 4 are the cell lysate
of the **TNF-gamma** and the control.

Exa=1e 3
E2Mression of Recombinant TNF-cFamma in COS cells
The expression of plasmid, **TNF-gamma** HA is derived from
a vector pcDNAI/Amp (Invitrogen) containing: 1) SV40 origin of replication, 2) ampicillin resistance gene, 3) E.coli replication origin, 4) CKV promoter followed by a polylinker region, a SV40 intron and polyadenylation site. A DNA fragment encoding the entire **TNF-gamma** precursor and a HA tag
fused in frame to its 3f end was cloned into the polylinker region of the vector, therefore,. . .

The DNA sequence encoding **TNF-gamma**, ATCC # 75927, was
constructed by PCR on the original EST cloned using two primers: the 5F primer (same as for the bacula example) contains a BamHI site followed by 24 nucleotides of **TNF-gamma**
coding sequence starting from the initiation codon; the 31 sequence 51 CGCTCTAGATCAAGCGTAGTCTGGGACGTCGTATGGATAGTAAGAAG GCTCCAAAG 3' contains complementary sequences to XbaI site, translation stop codon, HA tag and the last 18 nucleotides of the **TNF-gamma** coding sequence (not including the stop codon) .

Therefore, the PCR product contains a BamHI site, **TNF-**

gamma
coding sequence followed by HA tag fused in frame, a translation termination stop codon next to the HA tag, and an XbaI site.. . . DNA was isolated from transformants and examined by restriction analysis for the presence of the correct f ragment. For expression of the recombinant **TNF-gamma**, COS cells were transfected with the expression vector by DEAE-DEXTRAN method. (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the **TNF-gamma** HA protein was detected by radiolabelling and immunoprecipitation method.

Example 4
E2Wression pattern of TNF-qamma in human tissue
RNA blot analysis was carried out to examine the levels of expression of **TNF-gamma** in human tissues. Total cellular RNA samples were isolated with RNAzolm B system (Biotecx Laboratories, Inc. 6023 South Loop East, Houston, TX. . . . Maniatis, Molecular Cloning, Cold Spring Harbor Press, (1989)). The labeling reaction was done according to the Stratagene Prime-It kit with 50 ng **TNF-gamma** cDNA, to produce 32p -labeled **TNF-gamma** cDNA. The labeled DNA was purified with a Select-G-50 column (5 Prime - 3 Prime, Inc. 5603 Arapahoe Road, Boulder, CO 80303). The filter was then hybridized with radioactive labeled full-length **TNF-gamma** gene at 1,000,000 cpm/ml in 0.5 M NaPO4. pH 7.4 and 7% SDS overnight at 65*C. After being washed twice at. . . with 0.5 x SSC, 0.1% SDS, the filter was then exposed at -70*C overnight with an intensifying screen. The message RNA for **TNF-gamma** is abundant in kidney (Figures 3A).

. . .
3B, with the only change being that 10 Mg poly A RNA from the tissues indicated were used. The message RNA for **TNF-gamma** is expressed predominantly in HUVEC cells (Figure 3B).

Exa=le 5
Ability of Recombinant **TNF-gamma** to inhibit WEHI 164 and L929 cell crrowth, induce cell adhesion in HL-60 cells and promote endothelial cell growth.

. . .
plates containing 0.1 ml serially diluted test samples of cells (WEHI 164 and L929). Incubation was continued for 70 hours. TNF-a. TNF-fl and **TNF-gamma** were added at a 0.5 Ag/ml concentration. The cytotoxicity and proliferation activity was quantified using

an MTS assay performed by the addition. . . % cytotoxicity = (100 - OCexpen=nu1/ODc,,u,1) X 100. The photographs were taken after 72 hours. As shown by Figure 6A, and 8 **TNF-gamma** induced a morphology change which appeared as dark round cells which are killed.

. . . graph of Figure 6B. the assay was performed as described above, however, increasing amounts of TNF were added. The results indicate that **TNF-gamma** is an inhbitor of WEHI 164 cells.

To test adhesion ability of **TNF-gamma**, HL-60 cells were used and cell adhesion and cell-cell contact were measured by observation under the microscope and scored subjectively by two independent investigators. Figure 10 illustrates **TNF-gamma**'s ability for inducing cell adhesion.

In the assay to test for ability of **TNF-gamma** to promote endothelial cell growth, the proliferation index (PI) was calculated as follows: PI Mexmrml/Mcomrol. Figure 8 illustrates that **TNF-gamma** is a promotor of endothelial cell growth.

. . . (eg., blocking solution). During the second incubation step, the nucleosomes contained in the WEHI 164 cell sample treated with the TNF-a, TNF-O or **TNF-gamma** bind via their histone components to the immobilized anti-histone antibody. In the third incubation step, anti-DNA-peroxidase (POD) reacts with the DNA-part of the. . . are indicated as the absorbance A405nm/A490. (See Boehringer mannheim Catalogue, 0990 C 93 2 1541170) (see Figure 7)

Exanwle 7

Receptor binding assay using **TNF-gamma**

TNF-a and **TNF-gamma** were Ni-NTA affinity chromatography purified using the 6-His tag and I Ag/well was added to a nickel chelate coated 96-well plate (Xenopore. . .

. . . OD was measured using an ELISA reader (test wavelength 450 nm, correction wavelength 590 nm). The results shown in Figure 11 illustrate that **TNF-gamma** does not bind significantly to sTNF-receptors.

(ii) TITLE OF INVENTION: Human **Tumor Necrosis Factor-Gamma**

(iii) NUMBER OF SEQUENCES: 2

UV) CORRESPONDENCE ADDRESS.

CLMEN. . . cells with the

vector of Claim 9.
. An isolated DNA hybridizable to the DNA of Claim 2 and encoding a polypeptide having **TNF-gamma** activity.

19 A method for the treatment of a patient having need of **TNF-gamma** comprising: administering to the patient a therapeutically effective amount of the polypeptide of claim 14.

20 A method for the treatment of a patient having need of **TNF-gamma** comprising: administering to the patient a therapeutically effective amount of the agonist of claim 17.

21 A method for the treatment of a patient having need to inhibit **TNF-gamma** comprising: administering to the patient a
therapeutically effective amount of the antagonist of Claim 18.

. . .
polypeptide of claim 14 comprising:
combining endothelial cells, Concavilin-A, the compound to be screened, [3H] thymidine selectively in the presence or absence of **TNF-gamma;**
measuring the [3 HIthymidine incorporation by the endothelial cells; and
determining if the compound enhanced or blocked [3 HIthymidine incorporation.
. A method for inhibiting tumor cell growth in a patient comprising:
administering to the patient a therapeutically effective amount of the **TNF-gamma** protein optionally in the
presence of a pharmaceutically acceptable carrier.

. . .
A method for diagnosing a tumor or a susceptibility to a tumor comprising:
detecting a mutated form of the nucleic acid sequence encoding **TNF-gamma** in a sample derived from a host.

=>

L7 ANSWER 9 OF 13 MEDLINE
ACCESSION NUMBER: 2001175167 MEDLINE
DOCUMENT NUMBER: 21170294 PubMed ID: 10973284
TITLE: **APRIL** and TALL-I and receptors BCMA and TACI: system for regulating humoral immunity.
AUTHOR: Yu G; Boone T; Delaney J; Hawkins N; Kelley M; Ramakrishnan M; McCabe S; Qiu W R; Kornuc M; Xia X Z; Guo J; Stolina M; Boyle W J; Sarosi I; Hsu H; Senaldi G; Theill L E
CORPORATE SOURCE: Department of Inflammation, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320-1799, USA.
SOURCE: Nat Immunol, (2000 Sep) 1 (3) 252-6.
Journal code: D0G; 100941354. ISSN: 1529-2908.
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LANGUAGE: English
FILE SEGMENT: Priority Journals
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ENTRY DATE: Entered STN: 20010410
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AB We report that the tumor neurosis factor homolog APRIL (a proliferation-inducing ligand) stimulates in vitro proliferation of primary B and T cells and increases spleen weight due to accumulation of B cells in vivo. APRIL functions via binding to BCMA (B cell maturation antigen) and TACI (transmembrane activator and CAML-interactor) and competes with TALL-I (also called BLyS or BAFF) for receptor binding. Soluble BCMA and TACI specifically prevent binding of APRIL and block APRIL-stimulated proliferation of primary B cells. BCMA-Fc also inhibits production of antibodies against keyhole limpet hemocyanin and Pneumovax in mice, indicating that APRIL and/or TALL-I signaling via BCMA and/or TACI are required for generation of humoral immunity. Thus, APRIL-TALL-I and BCMA-TACI form a two ligands-two receptors pathway involved in stimulation of B and T cell function.

L7 ANSWER 10 OF 13 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 1998416181 MEDLINE
DOCUMENT NUMBER: 98416181 PubMed ID: 9743536
TITLE: **APRIL**, a new ligand of the **tumor necrosis** factor family, stimulates tumor cell growth.
AUTHOR: Hahne M; Kataoka T; Schroter M; Hofmann K; Irmler M; Bodmer J L; Schneider P; Bornand T; Holler N; French L E; Sordat B; Rimoldi D; Tschopp J
CORPORATE SOURCE: Institute of Biochemistry, Lausanne Branch, University of Lausanne, Epalinges, Switzerland.
SOURCE: JOURNAL OF EXPERIMENTAL MEDICINE, (1998 Sep 21) 188 (6) 1185-90.
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AB Members of the **tumor necrosis** factor (**TNF**) family induce pleiotropic biological responses, including cell growth, differentiation, and even death. Here we describe a novel member of the **TNF** family designated APRIL (for a proliferation-inducing ligand). Although transcripts of APRIL are of low abundance in normal tissues, high levels of mRNA are detected in transformed cell lines, and in human cancers of colon, thyroid, and lymphoid tissues in vivo. The addition of recombinant APRIL to various tumor cells stimulates their proliferation. Moreover, APRIL-transfected NIH-3T3 cells show an increased rate of tumor growth in nude mice compared with the parental cell line. These findings suggest that APRIL may be implicated in the regulation of tumor cell growth.